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Study of molecular response to radiations in Caenorhabditis elegans

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学位記番号	生博第230号
学位授与年月日	平成２４年３月２７日
学位授与の要件	学位規則第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）生態システム生命科学専攻
論文題目	Study of molecular response to radiations in *Caenorhabditis elegans*（*Caenorhabditis elegans* を用いた放射線に対する分子応答の研究）
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論文内容の要旨

Introduction

Nowadays, there are many kinds of radiations in our everyday life. These invisible rays are generally possible to divide in two different groups. One is non-ionizing radiation (NIR) and the other is ionizing radiation (IR). In this study, to examine the effects of these two radiations on living organisms to examine at molecular level, I used an experimental model animal *Caenorhabditis elegans*, which is easy to handle, genetically well characterized, and whose development and life cycle are well understood. Transcriptional alterations in response to one of NIR, static magnetic fields (SMFs) and IR were investigated by DNA microarray analysis with the whole-genome *C. elegans* GeneChip. I also constructed bioassay to monitor the effect of high SMFs and IR on DNA double-strand break (DSB) formation, because there are still arguments that SMFs may trigger DSB and genotoxicity. I also found a mammalian Mucin-like gene *F49F1.6* (termed *mul-1*) strongly induced by IR irradiation, which had been reported as one of the up-regulated genes following the infection of *Pseudomonas aeruginosa* (PA14). Therefore, I studied transcriptional signaling, mechanism, and biological function of the *mul-1* gene and would like to discuss a cross-talk between innate immune response and IR response.

Results

Different transcriptional alterations in exposures to SMFs and IR

As compared with the overall frequency of transcripts that exhibited increases due to high SMF exposure for 4 h, the frequencies of up-regulated genes in several categories were higher: actin binding, motor activity, cuticle, cell adhesion, and Ca^{2+} binding genes. In contrast, the apoptosis inducers genes, glutathione S-transferase-like genes, secreted surface protein genes including *mul-1* and other innate immune response genes were specifically induced by IR. These results indicate that the global transcriptional responses following high SMF exposure and IR are completely distinct.

Little genotoxicity by high SMF exposure

In the wild-type *C. elegans* hermaphrodites (XX), IR irradiation caused slightly increasing male (XO) progeny by a genotoxic effect via chromosomal instability (Fig. 1A). The *him-17* mutant hermaphrodite (XX) has high incidence of male (XO) progeny, because the mutant shows a defect of chiasmata formation

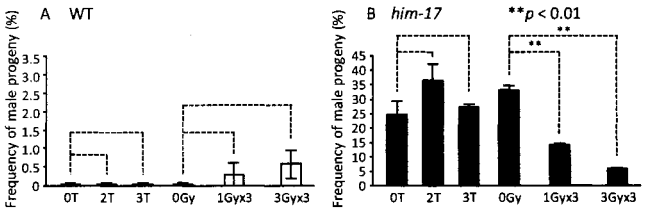

Fig. 1 Effect of SMFs or X-ray irradiation on the Generation of Male Progeny

via reduction of SPO-11 endonuclease activity which increases chromosomal nondisjunction during meiosis. DSBs induced by IR exposure could rescue the chromosomal nondisjunction and it results in oppositely decreasing frequency of male progeny (Fig. 1B). In contrast, high SMFs did not change the male production rate in either wild-type or *him-17* mutant background, indicating that genotoxicity by SMFs was little in the nematode.

Genes commonly up-regulated in IR and innate immune responses

mul-1 is significantly up-regulated both IR and bacterial infection. Some genes showed same tendency (Table 1).

Table 1. Up-regulated Genes by Exposure to IR or Bacterial Infection

Gene	Description	Fold change by IR	Fold change by infection
mul-1	Secreted surface	24.3	17.6
clec-4	C-type lectin	15.8	11.1
clec-67	C-type lectin	9.86	4.1
lys-1	Lysozyme	2.22	8.4
lys-2	Lysozyme	2.04	13.5

I irradiated the worms with 100 Gy X-ray for three times before seeding on lawns of PA14 and calculated the survival rate. As a result, pre-exposure to IR caused significant increase of survival rate compared with mock irradiations (Fig. 2). It suggests that pre-exposure to IR confers resistance to PA14 as an induction of adaptive response.

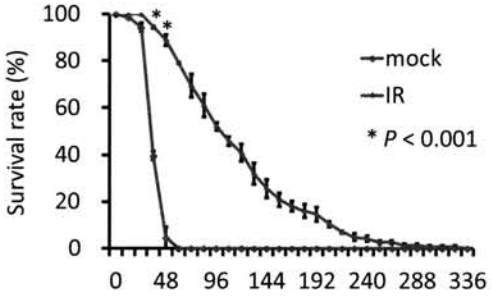

Fig. 2 Kinetics of the killing of *C. elegans* by *P. aeruginosa* after X-ray irradiation.

***mul-1* functions as an intestinal barrier protein**

I induced gene silencing of *mul-1* and observed the growth after exposure to IR. In the control, without irradiation, the growth rate was not altered by the depletion of *mul-1*. On the other hand, compared with mock RNAi, *mul-1* RNAi animals showed more severe growth retardation 3.5 and 6 days after irradiation (Fig. 3). Next, I used a 3MV tandem accelerator to irradiate the worm's intestine. Consequently, irradiation to intestinal surface caused up-regulation of *mul-1*. These results suggest *mul-1* functions as an intestinal barrier protein.

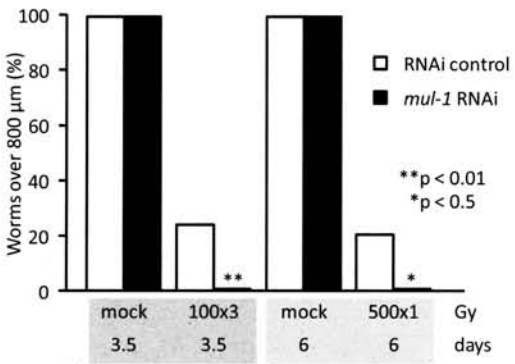

Fig. 3 Percentage of Worms Growing to Over 800 µm in Length

Transcription factors and signal transduction pathways involved in regulation of *mul-1*

I found DNA binding sequences for the GATA and FOXO transcription factors in the promoter region of the *mul-1* gene. ELT-2 is a GATA transcription factor involved in intestinal differentiation,

and DAF-16 is a FOXO transcription factor of the insulin/IGF-1 pathway which regulates a variety of genes involved in many stress responses. In the *elt-2* RNAi animals, induction of *mul-1* by IR was completely reduced. Moreover, *daf-16* mutant animals increased *mul-1* expression but to a much lesser degree than wild-type animals.

Next, to elucidate the signal transduction pathway by stress activated MAP kinases on *mul-1* induced by IR exposure, I used *C. elegans* p38 and JNK-1 signaling mutants. As a result, the induction of *mul-1* was completely inhibited in the p38 pathway mutants as well as other innate immune response genes tested.

Conclusion

In this study, I found that molecular responses to one of NIR, high SMFs, and IR exposures are completely different from each other. In addition, SMFs do not introduce any or few DSBs, which are lesions that are frequently induced by IR. In particular, magnetic resonance imaging (MRI) with high SMFs has become widely used for medical imaging purposes. My research is a result that supports the safety of the MRI unit.

I also found that the commonality of response to bacterial infection (an innate immune response) is only identified in IR. The tolerance to PA14 conferred by IR implies that a hormesis effect or an induction of adaptive response. Moreover, there was a cross-talk response at the transcriptional level between IR and innate immune response. From the results of *mul-1* gene regulation, the key molecules of this cross-talk were GATA, FOXO, and stress activated MAPK p38, which are highly conserved among species.

論文審査結果の要旨

放射線は、非電離放射線と電離放射線に大別できる。非電離放射線が生体へ与える影響は多くが明らかにされておらず、とりわけ遺伝毒性や DNA の二本鎖切断（DSB）が議論されていたが、未だ明確な結論には至っていなかった。一方で、電離放射線（IR）が生体へ与える影響については、DNA 修復、アポトーシスと行った分野の研究が盛んであるが、放射能泉の健康効果やホルミシス効果といった、IR がもたらす有益な影響についてはほとんど研究がされていない。そこで、本研究では、非電離放射線の一種である直流強磁場（SMF）と IR が生体に与える影響について、遺伝子発現レベルで比較解析を行い、加えて SMF については遺伝毒性・DSB が誘導されるか評価し、IR については、IR が生体へもたらす有益な影響について、モデル生物の一種である線虫（*C. elegans*）を用いて明らかにした。

線虫を SMF と IR にそれぞれ曝露し、全ゲノム DNA マイクロアレイを行った結果、SMF と IR では遺伝子の発現変動パターンが異なり、また、発現が有意に 2 倍以上上昇していた遺伝子群にも違いがみられた。この事から、両者が生体へ与える分子標的は異なっていることが示唆された。更に、新規に構築したバイオアッセイ系を用いて遺伝毒性・DSB について評価した結果、IR では共に検出できたが、SMF では検出されなかった。従って、これまで議論されてきた SMF による遺伝毒性・DSB に対し、それらは生じないと結論付けた。

IR を照射した DNA マイクロアレイの結果を更に精査したところ、新たに自然免疫応答遺伝子群が発現上昇していることを見出した。そこで、IR を照射後、緑膿菌を線虫に感染させた結果、IR 処理区では非処理区と比べて、緑膿菌の耐性が見られた。即ち、IR によるホルミシス効果により、緑膿菌への耐性が増したと考えられる。次に、IR と緑膿菌の両ストレスにおいて、共通して大きな発現上昇を示した F49F1.6 という遺伝子の機能・転写経路の解析を試みた。その結果、*F49F1.6* 遺伝子はヒトの分泌系ムチンと機能が類似している事を明らかにし、*mul-1* と命名した。また、IR の照射による発現誘導には p38　MAPK、ELT-2 が必須であり、インスリン IGF-1 経路は調節的に関与している事を明らかにした。

これらの成果は、自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって、木村孝文提出の論文は、博士（生命科学）の博士論文として合格と認める。